



# Evaluation of biofloc technology: the risk of giving different commercial probiotics to C:N and N:P ratio and quality of seawater

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**Abstract.** Biofloc technology is the solution to the problem of decreasing water quality related to aquaculture activities. The composition of the microflora in this technology is influenced by carbon, nitrogen and phosphorus through C:N and N:P ratios. This study examined the risks of different commercial probiotics for the ratio of N:P and C:N to biofloc media and the quality of seawater produced. This experiment was carried out with a completely randomized design (CRD) for 8 weeks with 3 treatments, namely probiotics Petrofish (A), Probiotics Pro I (B), and without probiotics (C). After 6 replications, the data were analyzed descriptively and statistically. After giving treatment, ratio of C:N was known in treatment A (2.46), B (1.83) and C (2.21) and N:P ratio in treatment A (22.01), B (22.08) and C (25.96). The treatment of different commercial probiotics in treatment A and treatment B of the ratio C:N and N:P was not significant ( $p > 0.05$ ). Seawater quality is based on pH parameters (7.94-8.11), dissolved oxygen (2.05-4.24 mg L<sup>-1</sup>) water temperature (26.5-27.1°C), salinity (22.33-25.17 ppt) and ammonia level (1-1.6 mg L<sup>-1</sup>). From the result, we emphasize that giving different commercial probiotics does not have an effect on the values of the C:N and N:P ratio and the seawater quality based on parameter is suitable to the standard.

**Key Words:** water management, seawater research, effectiveness of bioflox, environmental condition, aquaculture activities.

**Introduction.** Indonesia, one of the developing countries and an archipelago surrounded by the sea, enables the management of large natural and non-marine resources (FAO 2018). Development of aquaculture in Indonesia has enormous opportunities (Asmanah et al 2012). Developing countries become a source of 80 billion dollars in fishery exports, providing net trade income that is higher than the combined meat, tobacco, rice and sugar in 2016 (FAO 2016).

Global fish consumption per capita has increased to above 20 kilograms per year or doubled compared to the 1960s as a result of fisheries development (FAO 2018). Half of the seafood consumed globally comes from aquaculture. Aquaculture plays an important role in global food systems, the environment, and human health along with declining capture fisheries (Fry et al 2016). Aquaculture is one of the fastest growing food-producing sectors, supplying around 40% of fish food world (Cole et al 2012).

Apart from being beneficial to the community, this industry also has environmental degradation. Aquaculture practice arrangements, including site selection, pollution control, water quality, feed supply, and food security need to be done (Cole et al 2012; Martinez-Porchas & Martinez-Cordova 2012). Responsible fisheries governance, as well as national and international policies and laws will ensure sustainable harvests, maintain biodiversity and ecosystem functions, and adapt to climate change (Ekasari 2009; Garcia & Rosenberg 2015). So that, the result of deep sea water resources function as usual and can help overcome health problems (Nani et al 2016).

Water treatment in a bad cultivation system is one of the main obstacles that can lead to a decrease in water quality (Boyd & Green 2002). This reduction in water quality occurs due to waste from the rest of the cultivation activities. The remaining feed that is not eaten and feces will settle on the bottom of the water and is broken down by bacteria

into nitrogen and ammonia compounds. The amount of uncontrolled ammonia compounds in the waters can endanger cultured organisms (Avnimelech 2007; Ekasari & 2009).

The biofloc technology (BFT) application offers benefits in increasing aquaculture production that can contribute to the achievement of sustainable development. This technology can produce higher productivity with a smaller impact on the environment that can be developed and carried out in integration with other food production. This system is productively integrated to produce more food and feed from the same area of land with fewer inputs (Bossier & Ekasari 2017). In addition, BFT is one of the most beneficial alternative solutions to waste cultivation problems because it can reduce inorganic nitrogen waste (Crab et al 2007).

Probiotic parts cannot be separated from biofloc technology. The main principle applied in biofloc technology is management of water quality based on the ability of heterotrophic bacteria to utilize organic and inorganic N contained in the waters. (Purwanta & Firdayati 2002; Ekasari 2009; Gunarto & Suwoyo 2011). The principle of BFT is to grow microorganisms, especially heterotrophic bacteria in water conserving media. This growth is intended to absorb pollutant components, such as ammonia in the waters therefore this efforts can improve quality and benefit in environment. Heterotrophic bacteria will assimilate ammonia and nitrogen if the ratio of C:N to the media is well balanced (Schneider et al 2005). The application of BFT allows at least zero water to exchange practices during the cultivation period, and thus can increase sustainability, biosafety and production. Manipulating C/N ratio inputs has a significant influence on the development and characteristics of biofloc in zero-exchange systems by influencing inorganic nitrogen and phosphorus dynamics, and ultimately affecting performance (Xu et al 2016).

In addition to the C:N ratio, high nutrient concentrations in the waters will affect water productivity. The composition between nutrient components, namely the N to P ratio is often referred to as redfield ratio (Makmur et al 2012). Nitrogen and phosphorus are the most considered because they are related to CO<sub>2</sub>. Nitrogen and phosphorus, which are macro nutrients, both have benefits as limiting nutrients for phytoplankton growth (Souther & Rissik 2009).

A research to improve the effectiveness of biofloc technology and the quality of water produced is important, considering that it can be used as a support for future aquaculture technology. This study aims to study the risk of different commercial probiotics on the ratio of C:N and N:P to biofloc technology and the quality of seawater produced.

**Material and Method.** This experimental study was carried out for 8 weeks during June to July in 2015 and located in the Laboratory of the Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, while the examination of N, P and C elements was carried out in the Environmental Quality Laboratory of the Faculty of Civil Engineering and Planning, Institut Teknologi 10 November, Surabaya.

Complete Randomized Design (CRD) was carried out with three treatments and six replications. The experimental design consisted of three treatments for each probiotic Petrofish and Pro-1 and without probiotics, namely treatments A, B, and C respectively, which were repeated six times so that there were 18 experimental units, namely: A1, A2, A3, A4, A5, A6, B1, B2, B3, B4, B5, B6, C1, C2, C3, C4, C5, C6. The research was carried out by the formation of biofloc with probiotic Petrofish, the formation of biofloc with probiotics Pro-1 and the formation of biofloc without probiotics as controls.

The stages of the research are largely carried out through the sterilization of tools and materials with liquid soap and chlorine, the making and breeding of small-scale biofloc in-door and the addition of probiotics. The biofloc characteristics used were aquariums measuring 40 x 20 x 35 cm<sup>3</sup> filled with 4.5 liters of sea water with 22 ppt salinity and 3 point aeration. In the aquarium, every 7 days there are several ingredients included, namely 230 grams of fine bran, 90 grams of fish flour, 4.5 grams of yeast, 10 grams of urea fertilizer, 5 grams of SP-36 fertilizer, 12 mL molasses, 4.5 grams of dolomite and probiotic lime 2 mg L<sup>-1</sup>.

The main parameter in this study is the C:N and N:P ratios. The Kjeldahl method was applied in nitrogen testing (Saez-Plaza et al 2013). The nitrogen content used in the ratio calculation is anorganic nitrogen in the form of nitrite, nitrate and ammonia. The method used for testing phosphate content is the spectrophotometric method by calculating the inorganic phosphate content of orthophosphate. The method applied in carbon testing uses the gravimetry analysis (Miyazawa et al 2000). All parameters were observed on a daily basis for 21 days. The degree of acidity (pH), dissolved oxygen (DO) and temperature were measured twice a day, at 6:00 and 17:00. Ammonia measurements were measured once a week together with salinity measurements. The pH was measured twice a day at 6:00 and 17:00. After all data have been collected, they were analyzed statistically and descriptively. Statistical analysis was done using ANOVA with SPSS 16 for Windows. The results can be concluded as significant if p-value < 0.05. This shows a significant difference in the use of probiotics for C:N ratio and N:P ratio on biofloc technology.

**Results.** The results of this study consisted of test data for the content of C, N, P elements as well as calculating the ratios of C:N and N:P to the seawater experiment. Water quality parameters were observed in the form of acidity (pH), DO, temperature, ammonia, and salinity during the study. From the experimental results, the average value of N content at the beginning of the study showed that the highest value in treatment B (4,362.69 mg L<sup>-1</sup>) and the lowest value in treatment A (2,892.79 mg L<sup>-1</sup>). However, the average value of N content at the end of the study showed that the highest value in treatment C (3,698.00 mg L<sup>-1</sup>) and the lowest value in treatment A (3,207.92 mg L<sup>-1</sup>). Calculation of the floc volume formed in treatment A (44.33 mL L<sup>-1</sup>) and B (33.33 mL L<sup>-1</sup>) showed higher than in treatment C (16.66 mL L<sup>-1</sup>).

Testing the value of P content at the beginning of the study showed that treatment A had the highest value of 291.43 mg L<sup>-1</sup> and the lowest treatment B (137.36 mg L<sup>-1</sup>). The average value of P content at the end of the study showed that treatment B had the highest value (155.48 mg L<sup>-1</sup>) and the lowest treatment C was 146.59 mg L<sup>-1</sup>.

C content testing at the beginning of the study showed the highest value was found in treatment B (6,900 mg L<sup>-1</sup>) and the lowest was in treatment C (5,948 mg L<sup>-1</sup>). At the end of the study the average value of C content showed that treatment C had the highest value (8,206.66 mg L<sup>-1</sup>) and the lowest in treatment B (5,442.00 mg L<sup>-1</sup>).

The C:N ratio was obtained from data of C and N content in the media, then compared to get the value of the ratio C:N. The results of the measurement of C:N ratio at the beginning of the study showed that the value of the treatment ratio A gave the highest result (2.3) and the lowest at treatment B (1.58). The final value of the C: N ratio in all treatments has increased. The highest value of the C:N ratio occurred in treatment A (2.46±1.73858) and the lowest in treatment B (1.83±0.78).

The N:P ratio value is obtained from the data of N and P content in the media, then compared to get the value of the ratio N:P. The results of the measurement of the N:P ratio at the beginning of the study showed the value of the ratio in treatment B gave the highest results of 31.76 and the lowest in treatment A (9.92). Value of N:P ratio on the last day of the study on treatments A and C showed an increase to 22.01±12.24 and 25.96±3.81 respectively, while in treatment B it decreased to 22.08±8.14.

Giving different probiotics namely petrofsh probiotics, pro 1 probiotics and control without probiotics to the ratio C:N (p: 0.662) and N:P (p: 0.678) showed a non-significant result (p > 0.05). Table 1 shows the average data for N, P and C content and C:N and N:P ratios.

Table 1

The content of N, P and C and the value of the ratio N:P and C:N

| T | N (ppm)  |          | P (ppm) |        | C (ppm) |          | N:P ratio |                    | C:N ratio |                   |
|---|----------|----------|---------|--------|---------|----------|-----------|--------------------|-----------|-------------------|
|   | Start    | End      | Start   | End    | Start   | End      | Start     | End                | Start     | End               |
| A | 2,892.79 | 3,207.92 | 291.43  | 153.8  | 6.670   | 5,881.66 | 9.92      | 22.01 <sup>a</sup> | 2.30      | 2.46 <sup>a</sup> |
| B | 4,362.69 | 3,231.62 | 137.36  | 155.48 | 6.900   | 5,442    | 31.76     | 22.08 <sup>a</sup> | 1.58      | 1.83 <sup>a</sup> |
| C | 3,478.50 | 3,698.28 | 138.68  | 146.59 | 5.948   | 8,206.66 | 28.08     | 25.96 <sup>a</sup> | 1.7       | 2.21 <sup>a</sup> |

Note: the values with the same superscript in the same column show results that are not significantly different.

**Quality of seawater.** In the beginning of the study, the pH value was classified as acid, which ranged from 5.72 to 5.86 but at week 2 the pH value in all treatments experienced increases ranging from 7.40 to 7.47. Until the end of the study at week 3 the pH value in all treatments was relatively stable ranging from 7.94 to 8.11.

In the first week, the highest DO content was found in treatment C (2.85 mg L<sup>-1</sup>) and the lowest was treatment B (2.56 mg L<sup>-1</sup>). In the second week the DO content increased in all treatments, namely treatment A (3.36 mg L<sup>-1</sup>), treatment B (2.89 mg L<sup>-1</sup>) and treatment C (2.91 mg L<sup>-1</sup>). At the end of the study, which was the third week, all treatments still experienced an increase in DO with the highest in treatment A (3.92 mg L<sup>-1</sup>) and the lowest was treatment C (3.68 mg L<sup>-1</sup>). During this study DO was in the range of 2.05 to 4.24 mg L<sup>-1</sup> so that it can be said to be normal based on existing references (Becerril-Cortés et al 2018).

The water temperature in the study is known in the range of 26.5-27.1°C. A higher range is obtained from the results of the salinity assessment with an average during the study ranging from 22.33 to 25.17 ppt. Ammonia measurements tend to fluctuate even though at the beginning until the study ended the value of ammonia content ranged from 1 to 1.6 mg L<sup>-1</sup>.

Dissolved oxygen and pH at week 3 on all treatments were within acceptable limits even though at the beginning both parameters as in lower value. Oxygen, ammonia levels and salinity are relatively stable according to the limits determined from the first week to the end of the study. The quality of seawater compared with acceptable standard presented in Table 2.

Table 2

Quality of seawater based on degree of acidity, dissolved oxygen, temperature, ammonia level and salinity

| Treatment  | Mean       |            |            | Acceptable range                        |
|--|------------|------------|------------|---|
|  | Week 1     | Week 2     | Week 3     |   |
| <i>Degree of acidity (pH)</i>                    |            |            |            |   |
| Treatment A                                      | 5.86±0.40  | 7.46±2.29  | 8.11±0.04  | 7-9.5 (Bhatnagar & Devi 2013);          |
| Treatment B                                      | 5.72±0.20  | 7.40±0.33  | 7.94±0.15  | 6.8-8 (Becerril-Cortés et al 2018)      |
| Treatment C                                      | 5.79±0.38  | 7.47±0.42  | 7.97±0.17  |   |
| <i>Dissolved oxygen (DO) (mg L<sup>-1</sup>)</i> |            |            |            |   |
| Treatment A                                      | 2.70±0.45  | 3.36±0.88  | 3.92±0.32  | 3-5 (Bhatnagar & Devi 2013);            |
| Treatment B                                      | 2.56±0.51  | 2.89±0.88  | 3.71±0.53  | Above 4.0 (ideal) and at least 60%      |
| Treatment C                                      | 2.85±0.40  | 2.91±0.77  | 3.68±0.29  | saturation (Becerril-Cortés et al 2018) |
| <i>Temperature (°C)</i>                          |            |            |            |   |
| Treatment A                                      | 27±0.43    | 26.8±0.34  | 26.8±0.26  | 15-35 (Bhatnagar & Devi 2013);          |
| Treatment B                                      | 26.7±0.16  | 27.1±0.16  | 26.7±0.32  | 28-30 (Becerril-Cortés et al 2018)      |
| Treatment C                                      | 26.8±0.18  | 26.5±0.37  | 26.8±0.31  |   |
| <i>Ammonia level (mg L<sup>-1</sup>)</i>         |            |            |            |   |
| Treatment A                                      | 1±0.27     | 1.5±0.54   | 1.25±0.54  | 1-1.6 (FAO 2002)                        |
| Treatment B                                      | 1±0.25     | 1.6±0.51   | 1±0.25     |   |
| Treatment C                                      | 1±0.27     | 1.6±0.51   | 1.3±0.54   |   |
| <i>Salinity (ppt)</i>                            |            |            |            |   |
| Treatment A                                      | 22.50±0.54 | 24.00±0.89 | 24.67±0.81 | 35 (Alkhudhiri & Hilal 2018);           |
| Treatment B                                      | 22.67±0.81 | 23.67±0.81 | 25.17±0.75 | 0-50 (Becerril-Cortés et al 2018)       |
| Treatment C                                      | 22.33±0.51 | 23.50±0.54 | 24.67±0.51 |   |

**Discussion.** Giving different probiotics does not have a significant effect on the C:N ratio and the N:P ratio in biofloc media. The effect of adding probiotics in biofloc is unclear, because the results depend on the concentration and type used, but most importantly, the impact on the microbiota community is developed in the biofloc system (Cienfuegos et al 2017). Although the bacterial community plays the most important role in recycling organic materials and metabolizing toxic nitrogen compounds, addition of probiotics does not improve water quality or productive responses from cultivation (Arias-Moscoso et al 2018).

Microbial control in biofloc technology leads to efficient material degradation of waste, efficient nitrification and through manipulation of the C:N ratio. This effort aims to facilitate control and recycling of nitrogen and multiply protein utilization (Luo et al 2013). A balanced of C:N ratio will contribute naturally to the formation and stabilization of heterotrophic microbial communities through the availability of nutrients. These microorganisms play three main roles in maintaining water quality by absorbing nitrogen compounds that produce microbial proteins in situ, nutrition increases cultural feasibility by reducing feed conversion ratios (FCR) and decreasing feed costs and competition with pathogens (Becerril-Cortés et al 2018).

The highest C:N ratio value of this study only reached 2.46 in treatment A, this value is still much lower than previous studies conducted in fresh water which is 7.8. (Yurika 2015). This is allegedly due to the use of culture media which different, namely sea water and fresh water. Nitrogen content in marine waters is higher than in freshwaters. Nitrogen is a biological inert gas and the problem of excess N<sub>2</sub> in water is supersaturation. This gas is often saturated in waters which pumped from groundwater. If air saturation increases by more than 110%, this saturation can cause problems in some fish, by causing "gas embolism" or gas bubble disease. This problem can increase the depth of dissolved gas content, therefore, it has implications in using pumped water from the depths for aquaculture purposes (FAO 2018)

As it is known, seawater has a more abundant variety of solutes, which are between 35,000 and 42,000 ppm (Biyantoro & Basuki 2007). Source of nutrient abundance in marine waters comes from various organic compounds carried from the land entering through river flow. Organic compounds that enter in waters will settle and undergo decomposition into inorganic compounds (Rousseau et al 2002). Inorganic compounds contain nutrients in the form of phosphates, nitrates and silicates which are important components for marine biota (Marojahan 2009).

The highest value of C:N ratio in this study is 2.46, this value is much lower compared to previous studies which ranged from 15 to 25 in making biofloc and attempted up to 20 (Amin & Abdul 2010; Gunarto & Suwoyo 2011; Husain et al 2014). Source C in this study comes from molasses and fine bran while for source N comes from fish flour and urea fertilizer. The use of these ingredients still cannot increase the value of the C:N ratio. The low value of the C:N ratio in this study is thought to be due to urea fertilizer as a source of N. Use of ZA fertilizer as a source of N can be used (Gunarto & Suwoyo 2011). The use of urea fertilizer in making biofloc is not recommended because it will be consumed directly by blue-green algae, and can therefore have an effect on blooming (Aiyushirota 2009). When bacteria are fed with organic substrates that contain mainly carbon and little or no nitrogen (sugar, starch, molasses, etc.), consequently they must take nitrogen from the water to produce proteins that needed for cell growth and multiplication. The low C:N ratio is related to the carbon state which functions as a limiting nutrient for heterotrophic bacteria populations in aquaculture ponds (Luo et al 2017).

Comparison of C and N is an important element that must be known in the formation of biofloc elements. The content of C at the end of the study revealed that treatment A and B experienced a decrease, indicating that the presence of probiotic bacteria in treatment A and B made use of element C. Element C is the main element that plays a role in the preparation of bacterial cells (Shewfelt et al 2005). Whereas in treatment C, the content of C experienced an increase because probiotic bacteria were not added to treatment media so that the C content in the media was not widely used by bacteria growing on the media. The contribution of bacteria to the use of carbon substrates relies on changes in bacterial biomass processing CO<sub>2</sub> into C in the pro glucose treatment process (Rinnan & Baath 2009). Increased ratio 10-20 of C:N, increases the volume of biofloc but the ratio 15 C:N, turns out to increase biofloc production better. The C/N ratio causes the best balance of better water quality, nutritional value C and resistance (Dauda et al 2018). If C:N ratio is optimal, BFT can be used to optimize the composition of the bacterial community for optimal water quality and optimal shrimp health. This study shows the possibility of obtaining better

performance than *Litopenaeus vannamei* culture by adjusting the C:N ratio correctly in biofloc based systems (Panigrahi et al 2018).

The N content in treatments A and C has increased while in treatment B has decreased. The increase in N content in treatments A and C shows that the process of reforming organic matter (fish and bran flour) becomes N. However, the increase in N elements cannot be utilized by probiotic bacteria so that the N content in treatments A and C does not show a decrease. While in treatment B, the N content in the media has decreased. It is suspected that in treatment B probiotic bacteria use N more quickly. Besides the use of probiotics B (Pro-1) which have a combination of *Bacillus subtilis* bacteria, *Bacillus licheniformis* and *Lactobacillus plantarum* are able to utilize N better. The high N content in treatment A is suspected because of the combination of the bacteria *Nitrosomonas* sp. and *Bacillus* sp. found in petrofish probiotics can degrade organic nitrogen compounds better so as to increase the content of inorganic N in the waters. *Nitrosomonas* sp. is a nitrifying bacteria which acts to oxidize ammonium to nitrate (Titiresmi & Sopiah 2006). *Bacillus* sp. is also able to degrade the organic nitrogen content into inorganic nitrogen and produce higher nitrate content (Irianto 2003). In productive systems with major biofloc technology elements for the growth of microorganisms derived from waste compounds from cultures of high density of species of interest for human consumption, which produce complex trophic networks in which nitrogen compounds become important molecules for the development of beneficial bacteria and food for culture (Jimenez-Ojeda et al 2018).

Sequentially the N content in treatment C had the highest content of 3698.28 mg L<sup>-1</sup> compared to other treatments. This is because the treatment of C is not given the addition of probiotic bacteria so that the growth of bacteria in the media is lower than that of treatments A and B. So that the N content in the media is not utilized optimally by bacteria, resulting in high N content in the C media, indicated by calculating the volume of floc formed. The N:P ratio at the end of the study ranged from 22.01 to 25.96. The value of the N:P ratio in this study is much higher than the study on fresh water which only reached 5.68 (Yurika 2015). This is presumably because the use of SP-36 fertilizer as a source of P and urea fertilizer as a source N can be utilized by blue-green algae and other microorganisms so that the value of the N:P ratio in the water becomes higher.

The highest value of the N:P ratio at the end of the study was seen in treatment C (25.96). The high value of the N:P ratio in treatment C shows that the N content in the media is relatively higher and the P content is lower. The increase in N content in treatment C was suspected because it was not done by adding probiotic bacteria so that N in the media was not utilized optimally compared to treatments A and B which were given the addition of probiotic bacteria. P element is one of the limiting factors for aquatic fertility that is closely related to phytoplankton composition (Reynolds et al 2001). The ratio of N:P will affect the autotroph community that will occur in the system (eg chlorophytes versus cyanophytes) (Becerril-Cortés et al 2018).

The P value in treatment C (146.59 ppm) was lower than in the other treatments. The low P value in this study is thought to be due to the phosphate content derived from sp-36 fertilizer. In addition, biofloc is a rich source of natural lipid-protein from food which available 24 hours per day because of the complex interactions between organic matter, physical substrate, and various types of microorganisms. This natural productivity plays an important role in recycling nutrients and maintaining water quality (Becerril-Cortés et al 2018).

Water temperature and plankton are water quality parameters that can affect the growth of phytoplankton. At the beginning of the study the pH measurements for each treatment were acidic, ranging from 5.72 to 5.86. This is thought to be due to the process of decomposition of organic matter by decomposing bacteria. In general seawater has a pH value greater than 7 and tends to be alkaline, but under certain conditions the value can be lower than 7 and acidic (Marojahan 2009). But at the end of the study the pH of all treatments experienced an increase of around 7.97-8.11. The pH value at the end of this study still meets the quality standard threshold for the cultivation of marine biota, which ranges from 7 to 8.5. The increase in pH value in this study is

suspected because the use of dolomite lime in the media can increase the pH content in all treatments.

The optimum temperature for the growth of microorganisms is around 28-32°C (Becerril-Cortés et al 2018). This is in accordance with the temperature measurement data in this study experiencing fluctuations which ranged from 26.7 to 26.8°C. The temperature will increase the chemical reaction in photosynthesis by 2-3 times. The increase in the rate of photosynthesis will continue to occur to a certain extent, because the temperature that exceeds the maximum limit will cause a denaturation of proteins and enzymes so that the metabolism in the cell stops (Makmur et al 2012). Temperature has good basis and consequences for metabolism and growth of organisms and the strength of interactions with the environment. The effects of temperature can be limited by changing the maintenance of zooplankton and the availability of nutrients combined with an increase in temperature. The temperature effects also differ for colonies from different compositions, different species of algae and different groups in their optimal temperature. Abiotic conditions affect the biomass of community phytoplankton exposed to different temperature conditions (Striebel et al 2016).

Organic materials in the media will be utilized and decomposed by decomposing bacteria to produce new microbial cells and compounds of CO<sub>2</sub>, NH<sub>3</sub>, H<sub>2</sub>S, and CH<sub>4</sub> as well as other compounds such as phosphorus and sulfide acid components. In this study biofloc began to appear formed in the second week. According to Yurika (2015), bioflocs begin to appear in the second week. Characteristics of the emergence of biofloc, namely the emergence of foam on the surface of the water, the color of the water is brown and gives off an unpleasant aroma. The characteristics of biofloc that have been formed are that there is abundant foam on the surface of the water (Gunarto & Suwoyo 2011). Media that have formed biofloc will be brown and pH value in the range of 7 (Aiyushirota 2009). This is thought to be due to the degradation of organic matter by decomposing bacteria that produce H<sub>2</sub>S compounds. As a result of the degradation process of organic matter in the media resulting in a decrease in DO content. This is in accordance with the results of measurements of DO at the beginning of the study which is relatively low, which is around 2.56-2.85 mg L<sup>-1</sup>. But at the end of the study the measurement of DO increased to 3.69-3.92 mg L<sup>-1</sup>. This shows that the effort to give aeration at 3 points in the media succeeded in increasing the value of DO even though the value was still relatively small.

Ammonia content values range from 1 to 1.6 mg L<sup>-1</sup>. Ammonia levels are very toxic at levels of less than 0.1 mg L<sup>-1</sup> even, they cause toxic effects on some fish. Tropical species can withstand higher toxicity and tropical fish ponds develop ammonia. Levels above 3-4 mg L<sup>-1</sup> are supported by carp and tilapia. Ammonia toxicity is mainly caused by ionized ammonia (UIA) (NH<sub>3</sub>). NH<sub>3</sub> (UIA) is 300-400 times more toxic than NH<sub>4</sub>. The toxicity effect of ammonia is high at high pH, the proportion of ionized ammonia is higher at higher pH. This aspect has indeed been well studied recently mainly because of its importance in intensive cultural systems (FAO 2002).

Biofloc technology (BFT), an aquaculture technique that is environmentally friendly, is based on microorganism production in situ. Continuous water movement in the water column is fully needed to induce macroaggregate formation (biofloc). BFT is considered a new "blue revolution" because nutrition can continue to be recycled and reused in culture media, benefiting from minimum exchange or zero water. Biofloc consumption by shrimp or fish has shown many benefits such as increasing growth rates, decreasing FCR, and associated costs in bait (Becerril-Cortés et al 2018). Results of measurements of seawater quality indicate that they are still within the threshold accepted by the environment. Maintenance and monitoring of water quality in aquaculture is an important practice that aims at the success of the growing cycle. Temperature, DO, pH, salinity, alkalinity, and orthophosphate are some examples of parameters that must be continuously monitored, especially in biofloc technology. Water quality parameters and their interactions in biofloc technology are very important for the development and maintenance of the correct production cycle. The safe range of these parameters will improve the quality of health growth and reduce mortality due to short-term and long-term eating patterns (Becerril-Cortés et al 2018). Biofloc technology has

many benefits for freshwater aquaculture. This technology provides a sustainable tool that is able to solve together the problems related to the environment, social aspects and the economy (Choo & Caipang 2015).

**Conclusions.** The use of different commercial probiotics had no effect on C:N ratio and N:P ratio. The quality of water produced is still in accordance with the standards for biota safety. Efforts are needed to increase the ratio of C:N by knowing the optimum dose of addition of molasses as a source of C as well as knowing that the amount of addition of SP-36 fertilizer is efficient to increase the value of the ratio N:P.

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